CHROMOSOME 19 FULL-INSERT CDNA SEQUENCING

Kimberly Lieuallen and Greg Lennon
Lawrence Livermore National Laboratory
Poster

The number of publicly available ESTs has gone up dramatically in the last year. However, the number of completely sequenced cDNA clones remains low since most EST sequence is derived only from the ends of the clones. In an effort to obtain complete sequence of chromosome 19 cDNAs, we have been evaluating both primer walking and transposon based sequencing methods. Primer walking is quite expensive and slow; the rate of the process is dependent on the speed at which oligonucleotides are designed, synthesized, and successfully employed. Recently our lab has switched to transposon sequencing. Selected chromosome 19 cDNAs from the LLNL-based I.M.A.G.E. Consortium collection have been subcloned into a modified pOT2 vector and conjugated with JGM cells to obtain transposons along the whole length of the cDNA. These clones are sequenced using M13 forward and reverse primers present on the transposon, and unambiguous double-stranded sequence from the full-insert of the cDNA is obtained. This method is superior to primer walking in terms of speed and quality of sequence. To date we have fully sequenced over 30 cDNAs, and will report on their characteristics.

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